Safety and efficacy of the HVTN 503/Phambili Study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study

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Summary

Background **The MRKAd5 HIV-1 gag/pol/nef subtype B vaccine was designed to elicit T-cell-mediated immune responses capable of providing complete or partial protection from HIV-1 infection or a decrease in viral load after acquisition. We aim to assess the safety and efficacy of the vaccine in South Africa, where the major circulating clade is subtype C.**

Methods **We did a phase 2b double-blind, randomised test-of-concept study in sexually active HIV-1 seronegative participants at five sites in South Africa. Randomisation was by a computer-generated random number sequence. The vaccine and placebo were given by intramuscular injection on a 0, 1, 6 month schedule. Our coprimary endpoints were a vaccine-induced reduction in HIV-1 acquisition and viral-load setpoint. These endpoints were assessed independently in the modified intention-to-treat (MITT) cohort with two-tailed significance tests stratified by sex. We assessed immunogenicity by interferon-γ ELISPOT in peripheral-blood mononuclear cells. After the lack of efficacy of the MRKAd5 HIV-1 vaccine in the Step study, enrolment and vaccination in our study was halted, treatment allocations were unmasked, and follow-up continued. This study is registered with the South Africa National Health Research Database, number DOH-27-0207-1539, and ClinicalTrials.gov, number NCT00413725.**

Findings **801 of a scheduled 3000 participants, of whom 360 (45%) were women, were randomly assigned to receive either vaccine or placebo. 445 participants (56%) had adenovirus serotype 5 (Ad5) titres greater than 200, and 129 men (29%) were circumcised. 34 MITT participants in the vaccine group were diagnosed with HIV-1 (incidence rate 4·54 per 100 person-years) and 28 in the placebo group (3·70 per 100 person-years). There was no evidence of vaccine efficacy; the hazard ratio adjusted for sex was 1·25 (95% CI 0·76–2·05). Vaccine efficacy did not differ by Ad5 titre, sex, age, herpes simplex virus type 2 status, or circumcision. The geometric mean viral-load setpoint was 20 483 copies per mL (n=33) in the vaccine group and 34 032 copies per mL (n=28) in the placebo group (p=0·39). The vaccine elicited interferon-γ-secreting T cells that recognised both clade B (89%) and C (77%) antigens.**

Interpretation **The MRKAd5 HIV-1 vaccine did not prevent HIV-1 infection or lower viral-load setpoint; however, stopping our trial early probably compromised our ability to draw conclusions. The high incidence rates noted in South Africa highlight the crucial need for intensified efforts to develop an efficacious vaccine.**

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Introduction

South Africa, the country with the largest number of people infected with HIV-1 (5·7 million)[1] and an HIV-1 prevalence of 11% in the general population,[2] is in dire need of a biomedical intervention to prevent HIV-1 infection. The MRKAd5 HIV-1 gag/pol/nef subtype B vaccine was designed to elicit T-cell-mediated immune responses that provided complete or partial protection from HIV-1 infection or a decrease in viral load after acquisition.[3–9] The adenovirus serotype 5 (Ad5) vaccine elicited immune responses in participants irrespective of Ad5 serostatus[10] with promising safety and immunogenicity data from earlier clinical studies.[10,11] The Step study[12] (the first phase 2b HIV vaccine test-of-concept study) was designed to assess the efficacy of this vaccine in regions of the world where the predominant circulating HIV subtype is clade B. The HVTN 503/Phambili (Zulu for "forward!") study was designed soon after the Step study as a test-of-concept study to assess efficacy in a clade C region of the world,[13,14] in populations with high levels of pre-existing immunity to Ad5.[15,16] Cross-clade T-cell immunity to HIV-1 *gag*, *pol*, and *nef* genes had been shown both in individuals infected with HIV-1 and in HIV-seronegative individuals vaccinated with clade B, providing an immunisation rationale for our trial in South Africa.[17,18] We stopped enrolment and vaccinations in our study in September, 2007, after the Step study's interim analysis, which reported that the vaccine did not protect against HIV-1 infection or reduce early viral load in those who acquired infection. In an exploratory analysis of Step data,

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the risk of HIV-1 infection also seemed to be higher in a subgroup of male vaccine recipients: those who were Ad5 seropositive or uncircumcised.[12] We report our findings on HIV-1 acquisition and disease progression as well as describe and investigate factors associated with HIV-1 acquisition as a means of guiding future biomedical interventions directed at reducing HIV-1 acquisition.

Methods

Participants

Between Jan 24, 2007, and Sept 19, 2007, we did a two-arm, double-blind, placebo-controlled randomised clinical trial at five sites within South Africa (Soweto, Cape Town, Klerksdorp-Orkney-Stilfontein-Hartbeesfontein, eThekwini, and Medunsa). We aimed to enrol predominantly heterosexual adults aged 18–35 years. Because of the generalised nature of the HIV-1 epidemic in South Africa, the only behavioural-risk eligibility criterion was being sexually active within the 6 months before enrolment. Pregnant or breastfeeding women were ineligible, and women had to agree to use two methods of contraception (barrier and another effective method, such as hormonal contraception) to avoid pregnancy from 21 days before their first vaccination until 1 month after their last vaccination.

On Sept 19, 2007, enrolment and vaccinations were halted on the basis of the interim analyses of the Step study,[12] and in October, 2007, unmasking of participants began, with concomitant HIV-1 testing, risk assessment, and counselling. After unmasking, follow-up visits were changed from every 6 months to every 3 months for more frequent HIV-1 testing and risk-reduction counselling, and these are ongoing.

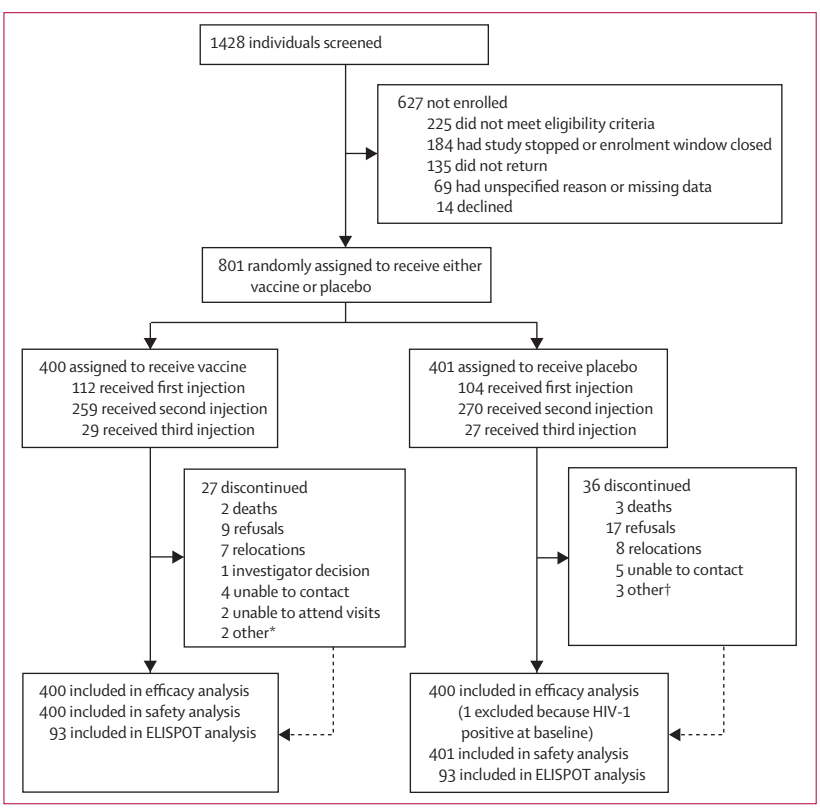

Figure 1: **Trial profile**
*Started on antiretroviral therapy. †Two started on antiretroviral therapy and one enrolled in another protocol.

Participants provided written informed consent in English or their local language. Our study was registered with the US Food and Drug Administration and approved by the South Africa Medicines Control Council, the Genetically Modified Organism Review Committee of the South Africa Department of Agriculture, and the ethical review committees and institutional biosafety committees of the University of the Witwatersrand, University of Cape Town, University of Limpopo, and the University of KwazuluNatal.

Procedures

Randomisation to vaccine or placebo (1:1) was stratified by site and sex. The randomisation sequence was on the basis of computer-generated random numbers and provided to site pharmacists by a central statistical and data monitoring centre. Treatment allocation was masked from the participants, study team, and laboratory personnel.

The MRKAd5 HIV-1 gag/pol/nef vaccine (Merck and Co Inc) has been described elsewhere,[12] but, in brief, the vaccine was given at a dose of $1{\cdot}5\times10^{10}$ adenovirus genomes per mL; the placebo was a 1 mL solution of the vaccine diluent with no Ad5 vector. The vaccine or placebo preparations were given by intramuscular injection on a 0, 1, 6 month schedule. Serum samples were obtained at enrolment for Ad5 neutralising-antibody titres[19] and herpes simplex virus type 2 (HSV2) serology.[20] We did clinical assessments and risk-reduction counselling at every visit. Before each vaccination, pregnancy was excluded. We obtained full blood count with differential, platelet counts, and alanine aminotransferase values 14 days after the first vaccination.

We assessed HIV-1 risk behaviours in the previous 6 months at screening and every 6 months until the termination of enrolment and vaccinations; after participant unmasking, these assessments were done every 3 months. At screening, we also assessed men for circumcision status by physical examination. We provided interventions to prevent HIV-1 to all participants throughout the trial, including risk-reduction counselling, provision of male condoms, partner and couple HIV testing and counselling, male circumcision,[21] management of sexually transmitted infections (STIs), and post-sexual exposure antiretroviral prophylaxis.

Before we stopped enrolment and vaccination, we did HIV-1 testing on blood drawn on the day of first vaccination, week 12, week 30, and every 6 months thereafter with an algorithm that distinguishes true infection from vaccine-induced seropositivity—an initial positive test result was confirmed by a second blood draw, which

	Men (n=441)		Women (n=360)		Total (n=801)
	Vaccine (n=222)	Placebo (n=219)	Vaccine (n=178)	Placebo (n=182)	
Adenovirus 5 titre					
≤18	50 (23%)	48 (22%)	25 (14%)	31 (17%)	154 (19%)
19–200	46 (21%)	55 (25%)	43 (24%)	58 (32%)	202 (25%)
201–1000	94 (42%)	95 (43%)	83 (47%)	62 (34%)	334 (42%)
>1000	32 (14%)	21 (10%)	27 (15%)	31 (17%)	111 (14%)
Age (years), median (range)	22 (18–35)	22 (18–35)	23 (18–35)	23 (18–34)	22 (18–35)
Race					
Black	222 (100%)	215 (98%)	176 (99%)	180 (99%)	793 (99%)
Other	0	4 (2%)	2 (1%)	2 (1%)	8 (1%)
HSV2 status					
Positive	40 (18%)	32 (15%)	90 (51%)	87 (48%)	249 (31%)
Negative	177 (80%)	181 (83%)	84 (47%)	93 (51%)	535 (67%)
Atypical	5 (2%)	5 (2%)	3 (2%)	2 (1%)	15 (2%)
Unknown	0	1 (0%)	1 (1%)	0	2 (0%)
Circumcised	61 (28%)	68 (31%)	..	..	129 (29%)
Study site					
Soweto-PHRU	82 (37%)	81 (37%)	72 (40%)	73 (40%)	308 (38%)
Cape Town	34 (15%)	31 (14%)	50 (28%)	51 (28%)	166 (21%)
KOSH	72 (32%)	72 (33%)	38 (21%)	39 (21%)	221 (28%)
CAPRISA	14 (6%)	15 (7%)	12 (7%)	12 (7%)	53 (7%)
Medunsa	20 (9%)	20 (9%)	6 (3%)	7 (4%)	53 (7%)
Risk behaviours (previous 6 months)*					
Number of sexual partners					
0	0	0	0	0	0
1	89 (40%)	105 (48%)	146 (82%)	158 (87%)	498 (62%)
2	60 (27%)	46 (21%)	29 (16%)	19 (10%)	154 (19%)
3–4	55 (25%)	52 (24%)	3 (2%)	5 (3%)	115 (14%)
≥5	18 (8%)	16 (7%)	0	0	34 (4%)
Median (range)	2 (1–20)	2 (1–14)	1 (1–3)	1 (1–3)	1 (1–20)
Serostatus of sexual partners					
Any HIV positive	4 (2%)	3 (1%)	2 (1%)	1 (1%)	10 (1%)
Any HIV unknown	163 (73%)	163 (74%)	87 (49%)	88 (48%)	501 (63%)
Any HIV negative	92 (41%)	90 (41%)	103 (58%)	99 (54%)	384 (48%)
Unprotected vaginal sex	129 (58%)	125 (57%)	103 (58%)	96 (53%)	453 (57%)
Unprotected receptive anal sex	0	1 (0%)	4 (2%)	5 (3%)	10 (1%)
Unprotected insertive anal sex	4 (2%)	8 (4%)	..	..	12 (3%)
Drinking or taking drugs with sex	93 (42%)	81 (37%)	26 (15%)	14 (8%)	214 (27%)
Had a main partner	155 (70%)	149 (68%)	151 (85%)	151 (83%)	606 (76%)
≥10 years younger (men only)	7 (3%)	4 (2%)	..	..	11 (2%)
≥10 years older (women only)	..	..	14 (8%)	13 (7%)	27 (8%)
Apart regularly	97 (44%)	95 (43%)	83 (47%)	74 (41%)	349 (44%)
Had a casual or anonymous partner	109 (49%)	100 (46%)	22 (12%)	18 (10%)	249 (31%)
Exchanged sex for money or gifts	12 (5%)	12 (5%)	2 (1%)	2 (1%)	28 (3%)
Forced to have sex	6 (3%)	3 (1%)	5 (3%)	2 (1%)	16 (2%)
Away from home regularly (men only)†	46 (21%)	48 (22%)	..	..	94 (21%)
Sexually transmitted infection‡	17 (8%)	10 (5%)	10 (6%)	9 (5%)	46 (6%)
Heavy drinking§	49 (22%)	59 (27%)	7 (4%)	9 (5%)	124 (15%)

*Behavioural-risk data are based on self-reported behaviour within 6 months before screening. †Apart regularly from main partner is defined as living in a different location or partner regularly away from home for 3 or more days per week, or, for men, being away from home for 3 or more days per week. ‡Sexually transmitted infection (STI) is based on participant self-report of an STI diagnosis. §Heavy drinking is defined as having more than five drinks per day on at least 10 days within the 6 month reporting period.

Table 1: **Baseline characteristics stratified by sex**

	Vaccine (n=400)	Placebo (n=401)	p value
Reactogenicity symptoms of any severity*			
Local pain or tenderness	247 (62%)	134 (33%)	<0·0001
Any systemic symptom	262 (66%)	220 (55%)	0·002
Headache	184 (46%)	149 (37%)	0·01
Malaise or fatigue	155 (39%)	118 (29%)	0·006
Myalgia	107 (27%)	61 (15%)	<0·0001
Arthralgia	82 (21%)	59 (15%)	0·03
Nausea	44 (11%)	38 (9%)	0·49
Chills	44 (11%)	28 (7%)	0·049
Diarrhoea	16 (4%)	19 (5%)	0·73
Vomiting	13 (3%)	18 (4%)	0·46
Adverse events†			
Participants with 1 or more adverse events	270 (68%)	270 (67%)	1·00
Upper respiratory tract infection	55 (14%)	54 (13%)	0·92
Headache	26 (7%)	15 (4%)	0·08
Influenza	19 (5%)	14 (3%)	0·38
Neutropenia	20 (5%)	10 (2%)	0·07
Hypertension	15 (4%)	14 (3%)	0·85
Genital discharge	15 (4%)	14 (3%)	0·85
Alanine aminotransferase increased	7 (2%)	4 (1%)	0·38
Haemoglobin decreased	8 (2%)	3 (1%)	0·14
Urinary tract infection	6 (2%)	5 (1%)	0·77
Pharyngitis	5 (1%)	4 (1%)	0·75
Skin laceration	5 (1%)	4 (1%)	0·75
Dizziness	6 (2%)	2 (0%)	0·18
Fungal skin infection	4 (1%)	3 (1%)	0·73
Oropharyngeal pain	4 (1%)	3 (1%)	0·73
Rash	4 (1%)	3 (1%)	0·73
Anaemia	5 (1%)	1 (0%)	0·12
Genital herpes	4 (1%)	2 (0%)	0·45
Leucopenia	4 (1%)	2 (0%)	0·45
Cough	4 (1%)	1 (0%)	0·22

*Reactogenicity symptoms were a set of specific symptoms commonly associated with vaccination that had an onset within the first 3 days after a study injection. †Adverse events were non-reactogenicity events that had an onset within 28 days after a study injection or were new chronic disorders needing medical intervention of more than 30 days, or newly diagnosed or treated sexually transmitted infections at any time during our study. Specifically listed adverse events are those with 1% or greater frequency in either the vaccine or placebo group and with the same or greater frequency in the vaccine group.

Table 2: **Reactogenicity symptoms and adverse events**

included HIV RNA detection. We tested for HIV-1 at unmasking and subsequently every 3 months. Participants infected with HIV-1 underwent physical examination, post-test counselling, and regular blood draws for monitoring of viral load and CD4 count, and were referred for medical care including antiretroviral therapy (ART).

We measured vaccine immunogenicity on a subset of participants in the HIV Vaccine Trials Network laboratory with a validated interferon-γ ELISPOT assay with two panels of peptide pools, clade B vaccine-matched and clade C potential T-cell epitopes, in previously cryopreserved peripheral-blood mononuclear cells.[22] We did the primary immunogenicity assessment on samples obtained by venepuncture at week 8, 4 weeks after the second vaccination from the first 186 participants enrolled (93 in the vaccine group and 93 in the placebo group) who were HIV-1 antibody negative at the week 12 visit, had received the second study injection, and whose thawed peripheral-blood mononuclear cells had 66% or greater cell viability.[23]

Our objectives were to assess the safety, tolerability, and efficacy of the MRKAd5 vaccine. We had two coprimary efficacy endpoints: acquisition of HIV-1 infection and HIV-1 viral-load setpoint in participants who became infected with HIV-1.

Statistical analysis

Our trial was event driven, designed to accrue at least 120 per-protocol events in 3000 participants to provide 80% power to detect a vaccine efficacy against infection of at least 45%, at least a 0·75 $\log_{10}$ copies per mL, or both reduction in the mean viral-load setpoints of participants in the vaccine group versus the placebo group. We defined vaccine efficacy as 100×(1–[vaccine infection rate/placebo infection rate]). We defined viral-load setpoint as the geometric mean of HIV plasma viral-load measurements (Roche COBAS Amplicor Monitor HIV-1 Standard, Roche Molecular Diagnostics, Pleasanton, CA, USA) at about 2 months to about 3 months after diagnosis. We used results from earlier timepoints when both 2 month and 3 month values were missing. We assessed our two endpoints independently with two-tailed significance tests stratified by sex. We applied a Hochberg multiplicity adjustment to the p values to adjust for the two endpoints.[24] Follow-up for HIV infection was to Aug 31, 2009, with viral load and CD4 data to Jan 15, 2010, to allow sufficient time to calculate viral-load setpoint.

Our efficacy endpoint analyses were modified intention-to-treat (MITT). Our original study design called for a per-protocol (PP) efficacy analysis, restricted to participants who received at least two vaccinations and were seronegative at the week 12 visit. However, because of the early stopping and consequent reduction in the PP and overall sample size, we changed our analysis plan to MITT before unmasking. The MITT population included all vaccinated participants apart from those diagnosed as infected with HIV-1 on the day of first vaccination.

Safety analyses included all participants randomly assigned to receive either vaccine or placebo who received at least one injection. Differences between treatment groups for reactogenicity and adverse events were assessed with Fisher's exact tests. Differences in STI rates were assessed with tests of homogeneity of the Poisson rates.

For HIV-1 infection, we used a Cox proportional hazards model to estimate the infection hazard ratio (HR) for vaccine to placebo adjusted for sex with Wald-based 95% CI. Time to HIV-1 infection for infected participants was defined as the time from first study injection to the midpoint between the last plasma HIV-1 RNA negative and first RNA positive test; for uninfected participants it was the time from first study injection to last day of study follow-up. Cumulative incidence plots of time to HIV-1

	Clade B vaccine matched			Clade C potential T-cell epitope		
	Ad5 antibody titre ≤18 (n=18)	Ad5 antibody titre >18 (n=75)	Overall (n=93)	Ad5 antibody titre ≤18 (n=18)	Ad5 antibody titre >18 (n=75)	Overall (n=93)
gag	16 (89%); 293	58 (77%); 192	74 (80%); 210	11 (61%); 220	37 (49%); 193	48 (52%); 199
pol	15 (83%); 837	50 (67%); 349	65 (70%); 427	15 (83%); 481	50 (67%); 216	65 (70%); 260
nef	13 (72%); 318	52 (69%); 184	65 (70%); 205	5(28%); 168	15 (20%); 148	20 (22%); 153
≥1 antigen (overall)	17 (94%); 1242	66 (88%); 587	83 (89%); 684	15 (83%); 787	57 (76%); 381	72 (77%); 443
≥2 antigen	15 (83%)	57 (76%)	72 (77%)	12 (67%)	35 (47%)	47 (51%)
≥3 antigen	12 (67%)	37 (49%)	49 (53%)	4 (22%)	10 (13%)	14 (15%)

Data are number (%); geometric mean of spot forming cells (SFCs) per 1 million peripheral-blood mononuclear cells for responders only, or number (%). Interferon-γ ELISPOT assay results are presented for the first 93 vaccinees enrolled into the trial who were HIV-1 plasma RNA negative at the week 12 visit, received the second vaccination within the visit window, had a week 8 blood draw within the visit window, and whose peripheral-blood mononuclear cell specimen had 66% or greater cell viability. For clade B, the pol magnitude is the sum of the SFCs for the two pol peptide pools. For clade C, the magnitudes for gag and pol are the maximum SFCs in the multiple peptide pools for the protein. The geometric mean for one antigen or more is based on the sum of the gene-specific magnitudes. Responders were defined as those with antigen-stimulated responses significantly greater than twice their background responses as assessed by a bootstrap test (one-sided α 0·05) after adjusting for the multiple antigens within each clade;[26] additionally, background-subtracted responses had to exceed ten SFC per 200 000 peripheral-blood mononuclear cells. In 93 placebo recipients assayed, four had a positive response for clade B and two of the four had a clade C response. The clade B peptide pools were synthetic peptide pools spanning the clade B proteins encoded by the vaccine constructs consisting of one gag, one nef, and two pol pools. Clade C peptide pools were: two gag PTE-C (potential T cell epitope-clade C), one nef PTE-C, and three pol PTE-C pools. Ad5=adenovirus serotype 5.

Table 3: **Interferon-γ-secreting T-cell responses by ELISPOT in vaccine recipients at week 8**

infection by treatment and sex are provided for illustration. To assess changes in the HR over time, we plotted the point estimate and 95% confidence bands for the instantaneous HR.[25] We used Cox proportional hazards models to assess other predictors of HIV-1 infection: age (in quartile groups), baseline Ad5 titre (both 18 and 200 cut-points assessed), number of vaccinations received (placebo group=0), baseline HSV2 seropositivity, baseline behavioural-risk factors (except for having an HIV-positive partner and exchange of sex, which could not be tested because of small numbers), baseline circumcision status (men), and use of hormonal contraceptives at baseline (women). We also used Cox models to assess time to CD4 decline to less than 350 cells per mL, a typical ART-initiation guideline.

For viral-load setpoint, values below the limit of detection (<400) were set to 400 copies per mL. We assessed the difference in the distributions of viral-load setpoint between vaccine and placebo groups with a Wilcoxon rank sum test stratified by sex. This study is registered with the South Africa National Health Research Database, number DOH-27-0207-1539, and ClinicalTrials.gov, number NCT00413725.

Role of the funding source

The study was reviewed by the Division of Acquired Immunodeficiency Syndrome of the US National Institute of Allergy and Infectious Disease, and the report was reviewed by both sponsors. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 1428 individuals screened, 801 were assigned to either treatment or placebo (figure 1). Of those enrolled, almost half were women and two-thirds were younger than 25 years (table 1). Nearly 20% of participants had baseline Ad5 antibody titres of 18 or less and 56% had Ad5 antibody titres greater than 200 (table 1). At enrolment, 129 men (29%) were already circumcised. An additional 52 men in the vaccine group and 57 in the placebo group were circumcised after enrolment—half before unmasking (26 vaccine and 29 placebo). HSV2 prevalence at baseline was significantly higher in women than in men (177 [49%] of 360 women *vs* 72 [16%] of 441 men; p<0·0001). Baseline demographic and HIV risk behaviours were similar between study groups except for women reporting drinking or taking drugs during sex in the 6 months before screening (p=0·04; table 1). Our cohort was predominantly heterosexual, with 457 (57%) of 801 participants reporting unprotected vaginal or anal sex as a risk factor. Men were more likely than women to report more than one sexual partner (247 [56%] of 441 men *vs* 56 [16%] of 360 women; p<0·0001) and to report having a casual or anonymous partner (209 [47%] of 441 men *vs* 40 [11%] of 360 women; p<0·0001). Most participants (304 [69%] of 441 men and 302 [84%] of 360 women) had a main partner, with 349 (58%) of these individuals with a main partner regularly living apart. Less than 10% of both men and women reported having a partner infected with HIV, exchanging sex for money or gifts, being forced to have sex, or being diagnosed with an STI, and few women reported heavy drinking (table 1).

Treatment groups did not differ in the number of injections received or in reasons for discontinuation of vaccination (figure 1). Overall, 63 participants (8%) withdrew from the study by Aug 31, 2009, the proportion being similar between treatment groups. After unmasking, more participants in the placebo versus vaccine group refused to further participate.

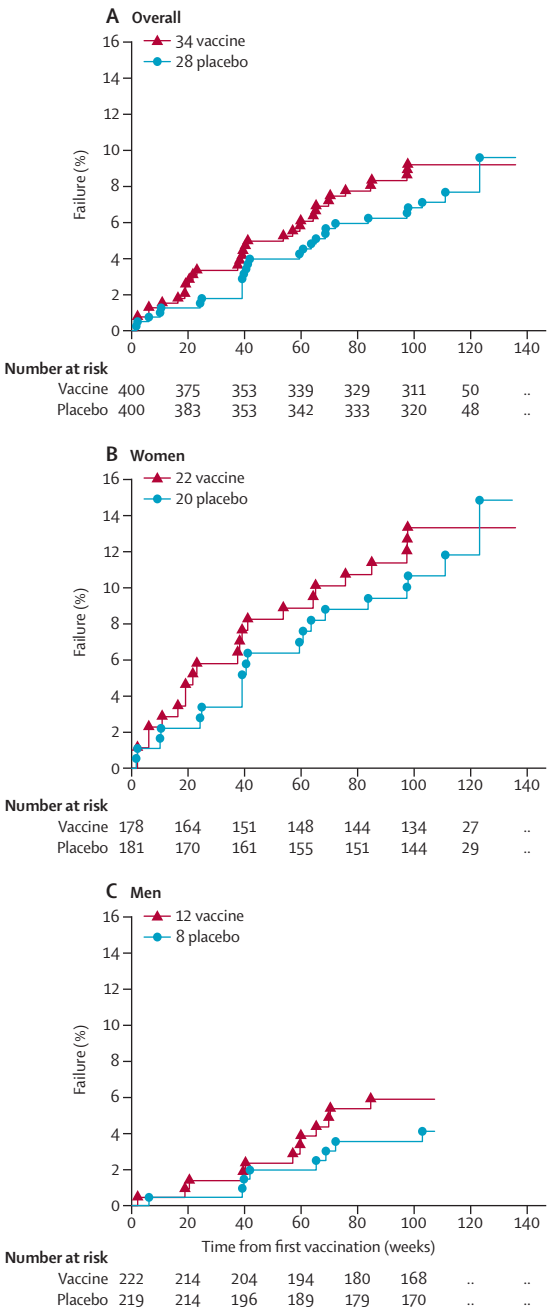

Figure 2: **Kaplan-Meier analysis of HIV-1 infection for vaccine and placebo groups**
Data are for the modified intention-to-treat cohort. One woman in the placebo group identified as infected with HIV at the time of first study injection is excluded from the cohort.

	Variable	HR contrast	HR	95% CI
Whole cohort	Treatment arm	Vaccine:placebo	1·31	0·79–2·16
	Age quartiles	18–20 years*	1	..
		21–22 years	3·28	1·47–7·32
		23–26 years	1·82	0·79–4·20
		27–35 years	1·95	0·85–4·46
Men	HSV2	Positive:negative	5·23	2·09–13·10
Women	HSV2	Positive:negative	1·00	0·53–1·89
HSV2 seronegative	Sex	Women:men	4·60	2·16–9·79
HSV2 seropositive	Sex	Women:men	0·88	0·41–1·88

The modified-intention-to-treat population includes all vaccinated participants apart from those diagnosed as HIV-1 infected on the day of first vaccination. Time to HIV-1 infection was calculated as the time from first study injection to last day of study follow-up for uninfected participants; for infected participants, from first study injection to the midpoint of the time between the last negative and first positive HIV-1 RNA PCR test. Estimates are from the final multivariate Cox proportional hazards model of time to HIV-1 infection, which included treatment group (Wald p=0·30), age quartiles (likelihood ratio p=0·03), sex (Wald p≤0·0001), baseline HSV2 status (Wald p<0·0004), and the interaction terms of sex × HSV2 (Wald p=0·0025). Adenovirus serotype 5 titre was not a significant predictor or effect modifier. HR=hazard ratio. HSV2=herpes simplex virus type 2. *Reference.

Table 4: **Risk factors for HIV-1 infection in the modified intention-to-treat population**

The vaccine was well tolerated (table 2) and the number of pregnancies in the two groups did not differ substantially (webappendix p 3). Self-reported STI rates were similar between vaccine and placebo groups over our entire study period (7·5 *vs* 9·8 per 100 person-years) as well as over the periods before and after unmasking (9·9 *vs* 9·6 and 7·7 *vs* 9·9 per 100 person-years). Women were more likely than men to report an STI (11·1 *vs* 6·7 per 100 person-years; p=0·007).

4 weeks after the second vaccination, most of the participants in the vaccine group in whom immunogenicity was tested had developed an interferon-γ-secreting T-cell response to clade B peptides and more than three quarters to clade C peptides (table 3). For clade B, gag-specific responses were highest; for clade C, pol-specific responses were highest. All clade C responders also had a clade B response. In responders to both clades B and C, the overall magnitude of response to the clade B vaccine-matched panel was significantly (p<0·0001) higher than to clade C potential T-cell epitope panel; the same pattern held for responses to individual antigens (table 3). Although not statistically different, vaccinees seronegative for Ad5 (titre ≤18) at baseline had consistently higher response rates for both clades, overall and by gene, than those who were seropositive for Ad5 (table 3). In responders, the Ad5-seronegative vaccinees also had a greater response than those who were seropositive for Ad5 for both clades overall (p=0·004 for B and p=0·007 for C), for clade B nef (p=0·049) and pol (p=0·002), and clade C pol (p=0·01).

There was no evidence of vaccine efficacy (figure 2): the treatment HR adjusted for sex was 1·25 (95% CI 0·76–2·05). Accounting for the time at which participants were notified of their treatment assignment did not alter this finding: the HR adjusted for unmasking time was 1·15 (95% CI 0·69–1·90). The treatment HR was higher in the 6 months (26 weeks) after first vaccination, but this finding was not significant (webappendix p 4). HIV incidence was similar in the

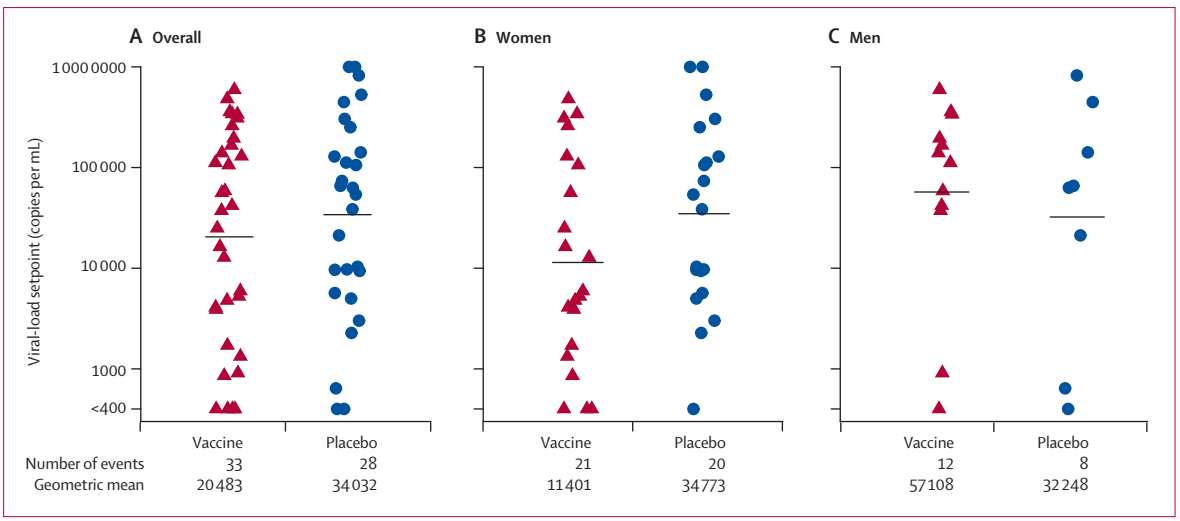

Figure 3: **Plasma viral-load setpoint by treatment group**
Viral-load setpoint is the geometric mean of the viral loads obtained at about 2 and 3 months after detection of infection. The bar denotes the geometric mean titres of the plasma viral load. One woman in the vaccine group was excluded because of a 6 month delay in confirming infection with HIV.

vaccine and placebo groups (4·54 *vs* 3·70 per 100 person-years, n=34 *vs* 28; webappendix p 1). Most of these infections were in women (n=42; figure 2) with high HIV-1 incidence rates both in the vaccine group (6·79) and placebo group (5·86).

In multivariate Cox proportional hazard models, sex, age, HSV2, and the interaction of sex and HSV2 were significant predictors of HIV-1 infection (table 4). Baseline Ad5 titre, categorised with either 18 or 200 as a cutpoint, was not a significant predictor of HIV-1 infection, and adjusting for Ad5 had little effect on the treatment HR (1·24–1·31, depending on model). The number of vaccinations and behavioural risk factors were also not significant predictors of infection. Ad5 titre, sex, age, or HSV2 did not modify the treatment HR, nor did the effect of age and Ad5 titre differ by sex.

HSV2 infection increased the risk of HIV-1 in men but not in women (table 4). For men, HSV2 increased the risk of infection more than five times. Of the participants who were HSV2 negative at baseline, women had a greater risk of infection with HIV-1 compared with men. When we restricted our analysis to women, there were no significant predictors of HIV-1 infection. In multivariable analysis for men (webappendix p 2), HSV2 (HR 4·90, 95% CI 2·03–11·80) and having a main partner but living apart regularly (HR 3·61, 95% CI 1·31–9·99) were associated with HIV-1 infection—neither Ad5 titre nor circumcision were significant. We did not assess HSV2 status after enrolment; in 31 participants who acquired HIV-1 and who were HSV2 seronegative at enrolment, 19 did not change HSV2 status and three acquired HSV2 before infection with HIV-1, and all nine remaining participants were identified as HSV2 and HIV-1 positive at the same visit.

We calculated viral-load setpoint for 61 of the participants infected with HIV-1. There was no significant difference in the distribution of viral-load setpoint between the vaccine group and the placebo group (p=0.39, stratified by sex; figure 3). Women tended to have lower viral-load setpoints than did men (geometric mean 19 642 *vs* 45 438 copies per mL), although the difference was not significant (p=0·15). Women who received vaccine tended to have lower viral-load setpoints than did women in the placebo group, although this difference was also not significant (p=0·16). Participants with baseline Ad5 titre greater than 18 (n=50) tended to have a higher viral-load setpoint than those with a titre of 18 or less (n=11; 31 924 *vs* 9924), but this too was not statistically significant (p=0·12).

Neither of the p values for our coprimary endpoints of HIV-1 infection and viral-load setpoint were statistically significant (each adjusted p=0·39). Three participants in the vaccine group and four in the placebo group started ART, all after the third month visit after diagnosis of infection with HIV-1. Two women with CD4 counts greater than 350 cells per mL were started on ART to prevent mother-to-infant transmission and data from these women were censored at 3 months and 1 year, respectively. Treatment was not a significant predictor of CD4 decline to fewer than 350 cells per mL overall or in men, but, in women, members of the vaccine group were at significantly lower risk of this event than those in the placebo group (HR 0·33, 95% CI 0·12–0·91; webappendix p 5). No other significant predictors of time to CD4 count less than 350 cells per mL were noted in the baseline covariates considered: site, Ad5 titre, age quartiles, HSV2, and their interactions with treatment.

See Online for webappendix

Panel: **Research in context**

Systematic review

We searched the PubMed database with the terms "MRK Ad5 gag/pol/nef HIV-1 vaccine", "Step study", and "HIV-1 vaccine trial". Our study is the second phase 2b randomised clinical trial that assessed the efficacy of the MRKAd5 HIV-1 gag/pol/nef vaccine in preventing acquisition of HIV infection or lowering viral-load setpoint in participants who became infected with HIV-1. The first trial (Step)[12] was done in North and South America, the Caribbean, and Australia where subtype B HIV-1 infection predominates, and in populations whose sexual risks were different (eg, men who have sex with men and heterosexual men and women at risk). In the Step study, the MRK Ad5 HIV-1 did not affect HIV-1 acquisition, or viral-load set-point and suggested a greater susceptibility to HIV-1 acquisition in subgroups of men who received vaccination (those who were Ad5 seropositive or uncircumcised). Our study was done solely in South Africa where subtype C HIV-1 infection predominates, where there is a higher rate of previous exposure to Ad5, and the participants were mainly sexually active heterosexual men and women.

Interpretation

Our findings support those of Step: the MRKAd5 HIV-1 gag/pol/nef vaccine had no effect on preventing HIV-1 acquisition (ie, no vaccine efficacy). We did not identify an association between Ad5 seropositivity or being uncircumcised and an increase in HIV-1 acquisition in those receiving the vaccine. This finding could be attributable to the early cessation of enrolment and vaccination, and possibly differences in risk behaviour. Post-hoc analyses of our study suggest trends of a vaccine effect in women who received the vaccine who subsequently became infected, toward lower viral-load setpoint and slower CD4 decline compared with women who received placebo. The importance of sex and route of HIV-1 transmission in vaccine clinical trials might warrant stratification.

Discussion

MRKAd5 HIV-1 vaccine does not prevent HIV-1 infection or lower early viral load in either Ad5-seropositive or Ad5-seronegative vaccinees; this finding is in accord with the findings of the Step study (panel). However, our study had restricted ability to assess the vaccine because of the discontinuation of enrolment and vaccination, possible changes in risk behaviour after unmasking, and the restricted number of infections particularly those that happened close to vaccination. This lack of vaccine effect is despite the high frequency and magnitude of HIV-1-specific T-cell responses measured by interferon-γ ELISPOT to both clade B and C antigens. Most vaccinees developed immune responses to both clades B and C, although the responses were greater to the vaccine-matched clade B peptides. Although the vaccine was immunogenic in our study and previous studies,[27] the responses elicited did not translate to vaccine efficacy.

The high incidence of HIV-1 in women permitted the first assessment of vaccine efficacy in heterosexual women and highlights the pressing need for effective preventive interventions for women. There was no evidence of increased risk of HIV-1 infection in vaccinated participants compared with placebo. There was also no such evidence in Ad5-seropositive women or men, or in uncircumcised men.

Although some of our results differ from those of Step, the premature interruption of our study, discontinuation of vaccinations, and unmasking might have affected risk behaviour, affected our statistical power to show associations, or might have altered susceptibility to infection. Furthermore, the men in our study were predominantly heterosexual, therefore risk factors would have differed from the predominantly homosexual population studied in Step.

Previous infection with HSV2 has been associated with greater infection with HIV-1[28–31] and was associated with greater HIV-1 acquisition in men who have sex with men in the Step study. In our study, previous infection with HSV2 was associated with infection with HIV-1 in heterosexual men but not women. The finding that HIV-1 risk was not associated with HSV2 in women has been documented previously.[32] The lack of association between oral or injectable hormonal contraception use at baseline and HIV-1 acquisition is consistent with other studies.[33] In men, HSV2 status and living apart from the main partner were associated with HIV infection, and interventions addressing these factors need to be studied.

The lower viral-load setpoint and slower CD4 decline noted in vaccinated women suggests a vaccine effect on disease progression, although, because enrolment was halted prematurely, our study was not powered to detect this effect. Pre-existing immunity to the vaccine vector might alter efficacy. Further studies are needed to understand the interplay between vaccination and these factors, including sex. A study of sexual partners of our study participants to provide new insights into the natural history of HIV infection and transmission dynamics in this population has been started.

Stratification by sex should be considered in future vaccine efficacy trials, to assesses the effects of HIV-1 vaccines on sex. Longer follow-up of incident infections is warranted to assess the effects of vaccination on disease progression.

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Contributors

ZM and BM did the analyses. GEG, MA, ZM, BM, GC, L-GB, MN, KM, GdB, MHL, MM, MR, JGK, and LC designed the study and wrote the report. DC, DKC, AP, and MJMc oversaw the laboratory, and conducted and interpreted laboratory data. GEG, GC, L-GB, NM, KM, GdB, SR, and NN did the Phambili study, oversaw the study, and managed the participants. GEG, MA, ZM, BM, LC, and JGK did the final editing.

Conflicts of interest

We declare that we have no conflicts of interest.

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References

1 UNAIDS. Report on the global AIDS epidemic. Geneva: Joint United Nations Programme on HIV/AIDS, 2008.

2 Shisana O, Rehle T, Simbayi L, et al. South African national HIV prevalence, incidence, behaviour and communication survey 2008: a turning tide among teenagers? Cape Town: HSRC Press, 2009.

3 Klein MR. Kinetics of Gag-specific cytotoxic T lymphocyte responses during the clinical course of HIV-1 infection: a longitudinal analysis of rapid progressors and long-term asymptomatics. *J Exp Med* 1995; **181:** 1365–72.

4 Harrer T, Harrer E, Kalams SA, et al. Strong cytotoxic T cell and weak neutralizing antibody responses in a subset of persons with stable nonprogressing HIV type 1 infection. *AIDS Res Hum Retroviruses* 1996; **12:** 585–92.

5 Kalams SA, Buchbinder SP, Rosenberg ES, et al. Association between virus-specific cytotoxic T-lymphocyte and helper responses in human immunodeficiency virus type 1 infection. *J Virol* 1999; **73:** 6715–20.

6 Shiver JW, Fu TM, Chen L, et al. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature* 2002; **415:** 331–35.

7 Shiver JW, Emini EA. Recent advances in the development of HIV-1 vaccines using replication-incompetent adenovirus vectors. *Annu Rev Med* 2004; **55:** 355–72.

8 Ramsburg E, Rose NF, Marx PA, et al. Highly effective control of an AIDS virus challenge in macaques by using vesicular stomatitis virus and modified vaccinia virus Ankara vaccine vectors in a single-boost protocol. *J Virol* 2004; **78:** 3930–40.

9 Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* 1997; **278:** 1447–50.

10 Priddy FH, Brown D, Kublin J, et al. Safety and immunogenicity of a replication-incompetent adenovirus type 5 HIV-1 clade B gag/pol/nef vaccine in healthy adults. *Clin Infect Dis* 2008; **46:** 1769–81.

11 Tobery TW, Dubey SA, Anderson K, et al. A comparison of standard immunogenicity assays for monitoring HIV type 1 gag-specific T cell responses in Ad5 HIV Type 1 gag vaccinated human subjects. *AIDS Res Hum Retroviruses* 2006; **22:** 1081–90.

12 Buchbinder SP, Mehrotra DV, Duerr A, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet* 2008; **372:** 1881–93.

13 Bredell H, Martin DP, Van Harmelen J, et al. HIV type 1 subtype C gag and nef diversity in southern Africa. *AIDS Res Hum Retroviruses* 2007; **23:** 477–81.

14 Woodman Z, Williamson C. HIV molecular epidemiology: transmission and adaptation to human populations. *Curr Opin HIV AIDS* 2009; **4:** 247–52.

15 Nwanegbo E, Vardas E, Gao W, et al. Prevalence of neutralizing antibodies to adenoviral serotypes 5 and 35 in the adult populations of The Gambia, South Africa, and the United States. *Clin Diagn Lab Immunol* 2004; **11:** 351–57.

16 Mast TC, Kierstead L, Gupta SB, et al. International epidemiology of human pre-existing adenovirus (Ad) type-5, type-6, type-26 and type-36 neutralizing antibodies: correlates of high Ad5 titers and implications for potential HIV vaccine trials. *Vaccine* 2010; **28:** 950–57.

17 Ferrari G, Humphrey W, McElrath MJ, et al. Clade B-based HIV-1 vaccines elicit cross-clade cytotoxic T lymphocyte reactivities in uninfected volunteers. *Proc Natl Acad Sci USA* 1997; **94:** 1396–401.

18 Coplan PM, Gupta SB, Dubey SA, et al. Cross-reactivity of anti-HIV-1 T cell immune responses among the major HIV-1 clades in HIV-1-positive individuals from 4 continents. *J Infect Dis* 2005; **191:** 1427–34.

19 Aste-Amezaga M, Bett AJ, Wang F, et al. Quantitative adenovirus neutralization assays based on the secreted alkaline phosphatase reporter gene: application in epidemiologic studies and in the design of adenovector vaccines. *Hum Gene Ther* 2004; **15:** 293–304.

20 Ashley RL, Militoni J, Lee F, Nahmias A, Corey L. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. *J Clin Microbiol* 1988; **26:** 662–67.

21 de Bruyn G, Martinson NA, Nkala BD, et al. Uptake of male circumcision in an HIV vaccine efficacy trial. *J Aquir Immune Defic Syndr* 2009; **51:** 108–10.

22 Malhotra U, Nolin J, Horton H, et al. Functional properties and epitope characteristics of T-cells recognizing natural HIV-1 variants. *Vaccine* 2009; **27:** 6678–87.

23 Dubey S, Clair J, Fu TM, et al. Detection of HIV vaccine-induced cell-mediated immunity in HIV-seronegative clinical trial participants using an optimized and validated enzyme-linked immunospot assay. *J Aquir Immune Defic Syndr* 2007; **45:** 20–27.

24 Hochberg Y. A sharper Bonferroni procedure for multiple tests of significance. *Biometrika* 1988; **75:** 800–02.

25 Gilbert PB, Wei LJ, Kosorok MR, Clemens JD. Simultaneous inferences on the contrast of two hazard functions with censored observations. *Biometrics* 2002; **58:** 773–80.

26 Moodie Z, Price L, Gouttefangeas C, et al. Response definition criteria for ELISPOT assays revisited. *Cancer Immunol Immunother* 2010; **59:** 1489–501.

27 McElrath MJ, De Rosa SC, Moodie Z, et al. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. *Lancet* 2008; **372:** 1894–905.

28 Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ. Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. *AIDS* 2006; **20:** 73–83.

29 Corey L, Wald A, Celum CL, Quinn TC. The effects of herpes simplex virus-2 on HIV-1 acquisition and transmission: a review of two overlapping epidemics. *J Aquir Immune Defic Syndr* 2004; **35:** 435–45.

30 Corey L. Synergistic copathogens—HIV-1 and HSV-2. *N Engl J Med* 2007; **356:** 854–56.

31 Weiss HA, Buve A, Robinson NJ, et al. The epidemiology of HSV-2 infection and its association with HIV infection in four urban African populations. *AIDS* 2001; **15** (suppl 4)**:** S97–108.

32 Ramjee G, Williams B, Gouws E, Van Dyck E, De Deken B, Karim SA. The impact of incident and prevalent herpes simplex virus-2 infection on the incidence of HIV-1 infection among commercial sex workers in South Africa. *J Aquir Immune Defic Syndr* 2005; **39:** 333–39.

33 Morrison CS, Richardson BA, Mmiro F, et al. Hormonal contraception and the risk of HIV acquisition. *AIDS* 2007; **21:** 85–95.